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Circular Dichroism of the β-Lactoglobulins between 190 and 320 nm

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THE technique of optical rotatory dispersion (ORD) has been used in the protein field for a number of years because of the information it can give on structural analysis, that is, on the α-helical and random structured portions existing in a protein in solution. With the present availability of sensitive instruments for measuring circular dichroism (CD) in the ultraviolet range, this latter technique has proven to be quite powerful, for although theoretically the same information is obtainable as that from ORD, the discrete character of the CD phenomenon improves resolution and allows increased accuracy.

Figure 1 shows the CD spectrum given by two most common varieties of bovine β-lactoglobulin (β-A and β-B) from 193 to 250 nm. Differences in optical properties within this family of proteins are very small. Examination of this spectrum shows no unusual features, and on the basis of models of known secondary structural conformation,[1] namely α-helix, random structures and the pleated-sheet or β-conformation, no preponderance of any specific conformation can be readily detected. The positive extremum, crossover point and negative peak occur at 194 nm, 200–201 nm and 212–213 nm respectively in all the β-lactoglobulins. These positions can be contrasted with peak positions given by poly-L-lysine in the three above-mentioned conformational states as shown in Fig. 1 of reference 1.

Figure 2 shows, as the upper solid line, the CD spectrum of β-B in the range of tryptophan and tyrosine absorption. This spectrum is an average of five spectral scans at pH values between 1 and 5. Ellipticities are typically two orders of magnitude smaller than at the lower wavelengths of

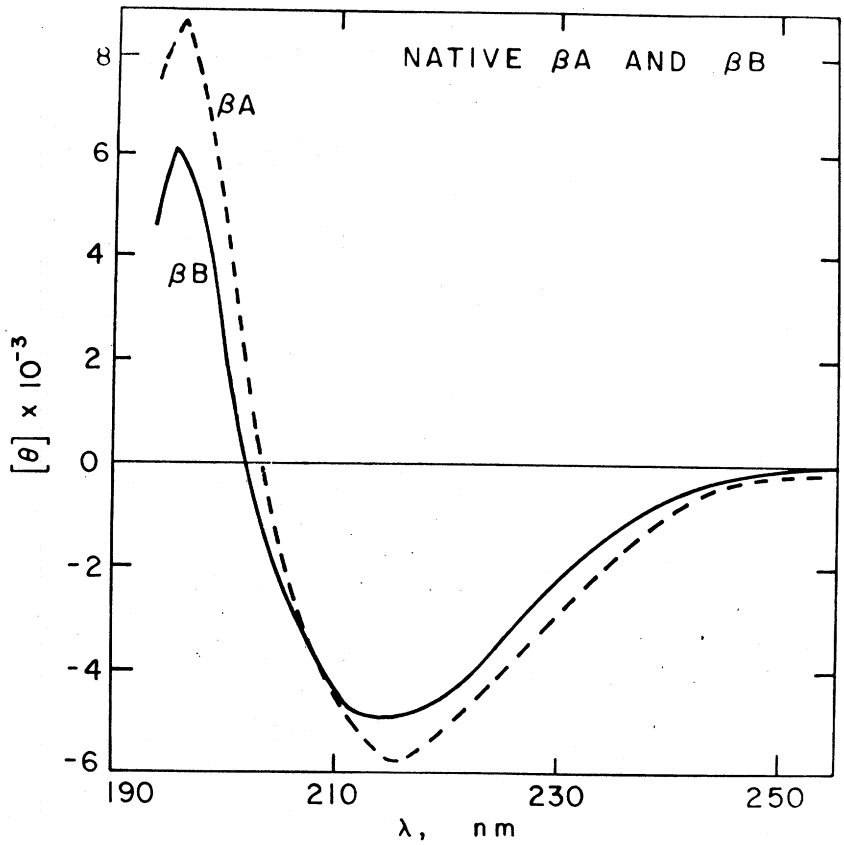

FIG.1. CD spectra of the two common varieties of bovie β-lactoglobulin below 250 nm.

Fig. 1. The spectrum is shown empirically decomposed into five peaks which occur at 270, 277, 285, 293 and near 300 nm. It is undoubtedly significant that in the cases of α-lactalbumin[2] and carbonic anhydrase,[3] ORD Cotton effects due to tryptophan have been reported at the two most intense peak wavelengths, 225 and 293 nm. CD peaks at these exact wavelengths are also apparent when films of tryptophan are examined.[4] It is likely that the two peaks at 227 and 270 nm are due to tyrosine residues, since optical activity at close to this wavelength has been found in ribonuclease.[5] The negative ellipticity seen (but not decomposed) between 255 and 265 nm may well be due to cystine or cysteine, as negative CD spectra have been reported for sulfur-containing peptides in exactly this spectral range.[6]

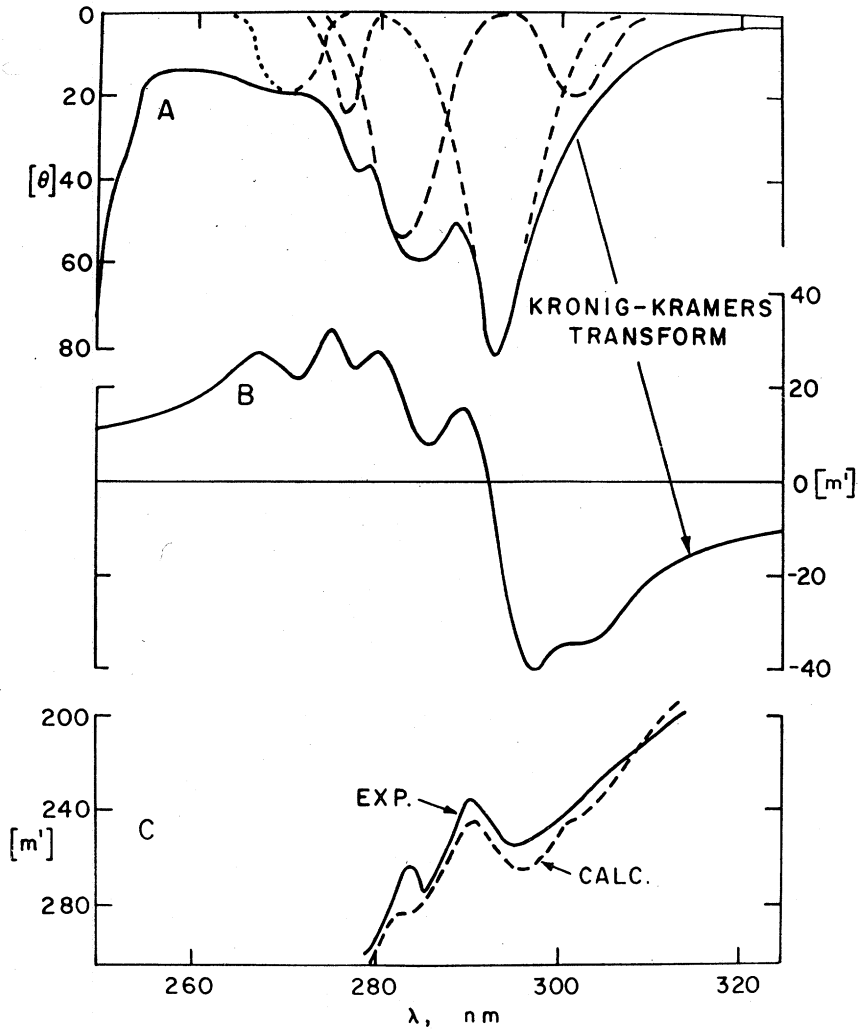

FIG. 2. (A) CD spectrum of β-B in region of aromatic absorption; dotted lines show decomposition. (B) Theoretical rotatory spectrum calculated from part A. (C) Dashed line: upper wavelength portion of part B added to estimated baseline (see text); solid line: experimentally determined rotations in this region.

A Kronig–Kramers transform computer program was applied to data taken from the decomposed curve and the resulting complex rotatory pattern is shown in part B of Fig. 2. Comparison of these calculated rota-

tions with experiment is somewhat difficult, since they are from 5 to 15 times smaller than the net protein laevo-rotation in this wavelength region. It was found possible, however, to plot the measured rotations from 250 to 265 nm, and from 300 to 320 nm, and to correct these for the summed aromatic rotation effects (part B, Fig. 2) since the latter values can be derived at any wavelength desired using the Kronig–Kramers transform. The resultant corrected rotations were joined by a smooth curve and the calculated values of part B were added to it through the central region. The sum is presented as the broken line in part C (Fig. 2) and is seen to be in good agreement with the experimental ORD data.

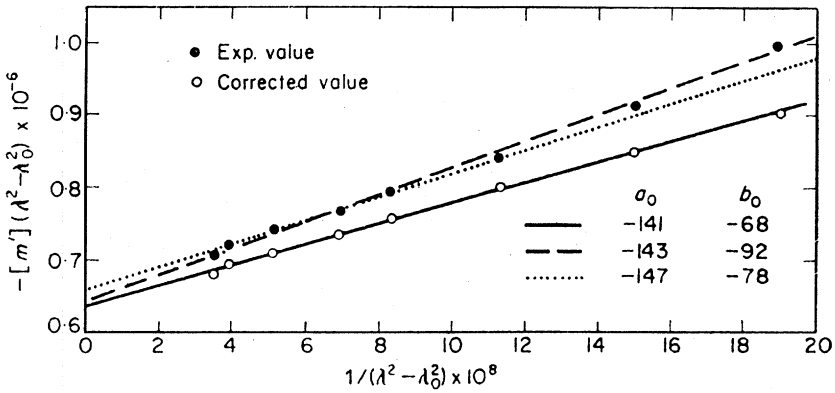

FIG. 3. Moffit–Yang plots of ORD data, and same data corrected for contributions of aromatic side chains. Dotted line shows effect of ignoring two lowest-wavelength points.

The result of the small negative dichroic bands is a complex rather broad "Cotton effect" (actually the sum of five negative Cotton effects) centered near 292 nm. Above this wavelength, rotations are more negative than they would be if this aromatic effect was not present. The effect of this enhanced rotation on the Moffit–Yang parameters a_0 and b_0 was then determined. These parameters are calculated from rotational data taken in the visible region (most commonly at the mercury arc wavelengths from 546 to 313 nm) and the usual plot of $m'(\lambda^2-\lambda_0^2)$ *versus* $1/(\lambda^2-\lambda_0^2)$ is made. Raw data, and data corrected for these aromatic Cotton effects are plotted in Fig. 3 for β-A. The b_0 is seen to be reduced almost 30%, while a_0 changes less than 2% (β-B shows corresponding changes: b_0 falls from -72 to -62, a_0 from -160 to -155. If the (two) lower wavelength

points, which curve up strongly when uncorrected, are neglected, as is shown by the dotted line, the results are still not correct, although somewhat closer to the corrected value. The importance of taking such aromatic rotatory contributions into account, if one is using a_0 and b_0 to perform a conformational analysis, should not be overlooked.

As a further point of interest, the corrected values of a_0 and b_0 may be used to calculate unperturbed rotations in the central portion of the aromatic range. When this was done for β-B, the calculated points fell exactly on the smooth curve constructed as a base-line to obtain part C, Fig. 2. This indicates that the electronic transitions of the polypeptide backbone which give rise to the major portion of the optical rotation, and which fix the λ_0 parameter at 212 nm, are sufficiently accounted for so that the Moffitt–Yang equation can correctly describe the dispersion of the rotation, in the case of the β-lactoglobulins, at least.

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